(b) The antiviral fluorochrome-conjugated antibodies to be used shall depend on the type of cells required to be tested for extraneous viruses as specified in an applicable Standard Requirement or in a filed Outline of Production. Antiviral fluorochrome-conjugated antibodies specific for the extraneous viruses shall be applied to each respective type of cell in accordance with the following list. Under certain circumstances, additional tests may need to be conducted, as determined by the Administrator. When a specific antiviral fluorochrome-conjugated antibody is used in testing for the listed extraneous viruses specified in more than one cell type, it need only be applied to the most susceptible cell type.

(1) All cells shall be tested for:
(i) Bovine virus diarrhea virus;
(ii) Reovirus; and
(iii) Rabies virus.

(2) Bovine, caprine, and ovine cells shall, in addition, be tested for:
(i) Bluetongue virus;
(ii) Bovine adenoviruses;
(iii) Bovine parvovirus; and
(iv) Bovine respiratory syncytial virus.

(3) Canine cells shall, in addition, be tested for:
(i) Canine coronavirus;
(ii) Canine distemper virus; and
(iii) Canine parvovirus.

(4) Equine cells shall, in addition, be tested for:
(i) Equine herpesvirus; and
(ii) Equine viral arteritis virus.

(5) Feline cells shall, in addition, be tested for:
(i) Feline infectious peritonitis virus; and
(ii) Feline panleukopenia virus.

(6) Porcine cells shall, in addition, be tested for:
(i) Porcine adenovirus;
(ii) Porcine parvovirus;
(iii) transmissible gastroenteritis virus; and
(iv) Porcine hemagglutinating encephalitis virus.

(7) Firms that do not have rabies virus on premises either for research or production purposes are exempt from having to produce positive rabies virus control monolayers. Fixed positive rabies virus control monolayers will be provided by the National Veterinary Services Laboratories.

(c) After staining, each group of monolayers shall be examined for the presence of specific fluorescence attributable to the presence of extraneous viruses.

(1) If the material under test shows any evidence of specific viral fluorescence, it is unsatisfactory and may not be used; *Provided*, That, if specific fluorescence attributable to the virus being tested for is absent in the positive control monolayers, the test is inconclusive and may be repeated.

(2) If the fluorescence of the monolayers inoculated with the specific virus as positive controls is equivocal, or if the negative monolayers show equivocal fluorescence indicating possible viral contamination, or both, the test shall be declared inconclusive, and may be repeated; *Provided*, That, if the test is not repeated, the material under test shall be regarded as unsatisfactory for use in the production of biologics.

[60 FR 24548, May 9, 1995]

INGREDIENT REQUIREMENTS

§ 113.50 Ingredients of biological products.

All ingredients used in a licensed biological product shall meet accepted standards of purity and quality; shall be sufficiently nontoxic so that the amount present in the recommended dose of the product shall not be toxic to the recipient; and in the combinations used shall not denature the specific substances in the product below the minimum acceptable potency within the dating period when stored at the recommended temperature.

[38 FR 29889, Oct. 30, 1973]

§ 113.51 Requirements for primary cells used for production of biologics.

Primary cells used to prepare biological products shall be derived from normal tissue of healthy animals. When prescribed in an applicable Standard Requirement or in the filed Outline of Production, each batch of

primary cells used to prepare a biological product shall be tested as prescribed in this section. A batch of primary cells found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from primary cells that are found unsatisfactory by any prescribed test.

(a) Final container samples of completed product or samples of the final pool of harvested material or samples of each subculture of cells used to prepare the biological product shall be shown free of mycoplasma as prescribed in §113.28. The sample for testing shall consist of at least 75 cm^2 of actively growing cells or the equivalent in harvest fluids; *Provided*, That all sources of cells in the batch of primary cells are represented.

(b) Final container samples of completed product or samples of the final pool of harvested material or samples of each subculture of cells used to prepare the biological product shall be shown free of bacteria and fungi as prescribed in §113.26 or §113.27 (whichever is applicable).

(c) A monolayer at least 75 cm^2 from each batch of primary cells or each subculture of primary cells used to prepare a biological product shall be shown free of extraneous agents as prescribed in this paragraph.

(1) The test monolayer shall be maintained using the medium (with additives) and under conditions similar to those used to prepare biological products.

(i) Monolayers of avian origin shall be maintained for at least 14 days and shall be subcultured at least once during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(ii) Monolayers not of avian origin shall be maintained for at least 28 days and shall be subcultured at least twice during the maintenance period. All but the last subculture shall result in a new monolayer of at least 75 cm^2. The last subculture shall meet the minimum area requirement specified in §§113.46 and 113.47.

(2) Monolayers shall be examined regularly throughout the required maintenance period for evidence of the presence of cytopathogenic agents. If evidence of a cytopathogenic agent is found, the batch of primary cells is unsatisfactory.

(3) At the conclusion of the required maintenance period, monolayers shall be tested for:

(i) Cytopathogenic and/or hemadsorbing agents as prescribed in §113.46;

(ii) Extraneous viruses by the fluorescent antibody technique as prescribed in §113.47.

[50 FR 442, Jan. 4, 1985, as amended at 60 FR 24549, May 9, 1995]

§113.52 Requirements for cell lines used for production of biologics.

When prescribed in an applicable Standard Requirement or in a filed Outline of Production each cell line used to prepare a biological product shall be tested as prescribed in this section. A cell line found unsatisfactory by any prescribed test shall not be used. A serial of biological product shall not be released if produced from a cell line that is found unsatisfactory by any prescribed test.

(a) *General requirements.* (1) A complete record of the cell line shall be kept, such as, but not limited to, the source, passage history, and medium used for propagation.

(2) A Master Cell Stock (MCS) shall be established at a specified passage level for each cell line. The passage level and identity of the MCS and the highest passage level (MCS + n) intended for use in the preparation of a biological product shall be specified in the Outline of Production for the product.

(3) Sufficient 1.0 ml or larger aliquots of MCS and MCS + n shall be prepared, kept in a frozen state, and made available to Animal and Plant Health Inspection Service (APHIS) upon request for performing the tests prescribed in this section.

(4) Each lot of cells shall be monitored for the characteristics determined to be normal for the cell line, such as, but not limited to, microscopic appearance, growth rate, acid